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# Pre- and perioperative factors affecting infection after living donor liver transplantation

## Risk factors affecting infection after liver transplantation

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the study, collected the data, analyzed the data and wrote the manuscript.  
AM, YO, KO, KH, AY, and SU performed the study.

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7  
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9 declare.

1   **Abstract**

2   **Objective:** Infectious complications including sepsis that often occur after  
3   liver transplantation (LT) comprise the most frequent causes of in-hospital  
4   death. This study investigated predictors of posttransplant infectious  
5   complications to establish a strategy with which to improve short-term  
6   outcomes after LT.

7   **Methods:** We used univariate and multivariate analyses to assess pre- and  
8   perioperative risk factors for posttransplant infectious complications among  
9   100 consecutive patients who underwent living donor LT between February  
10   2008 and February 2010 at our institute.

11   **Results:** Multivariate analysis showed that low preoperative body cell mass  
12   (BCM) and of the absence of preoperative supplementation with  
13   branched-chain amino acids were of prognostic significance for  
14   posttransplant sepsis. In addition, Child-Pugh classification C and massive  
15   operative blood loss were independent risk factors for posttransplant  
16   bacteremia and preoperative low BCM was an independent risk factor for  
17   in-hospital death due to infection.

18   **Conclusion:** Pretransplant nutritional intervention as well as a reduction in  
19   operative blood loss would help to prevent posttransplant infectious  
20   complications developing during living donor LT. BCAA supplementation  
21   before LT affects the occurrence of infectious complications.

22

- 1 **Keywords:** liver transplantation, infection, nutrition, body cell mass,
- 2 branched-chain amino acids.
- 3

## 1 Introduction

2 Infections after liver transplantation (LT) are the most frequent causes of  
3 morbidity and in-hospital death [1]. Patients who undergo LT are  
4 essentially regarded as being at unusually high risk for perioperative  
5 infection. For example, protein-energy malnutrition, which is common in  
6 patients with end-stage liver disease requiring LT, is considered to confer  
7 vulnerability to preoperative infection including spontaneous bacterial  
8 peritonitis and pneumonia via deteriorated immune function [2,3]. Liver  
9 transplantation itself is a massive invasion of the host. The number of  
10 intraoperatively transfused cellular blood products is also a risk factor for  
11 infections [4]. Furthermore, immunosuppression as well as multiple  
12 catheter insertion increases the risk of posttransplant infection.  
13 Consequently, infectious complications including sepsis and bacteremia  
14 often occur after LT and are the most frequent causes of in-hospital death.  
15 Therefore, the prevention of posttransplant infection plays a crucial role in  
16 improving short-term outcomes after LT.

17 Malnutrition is a risk factor for postoperative complications and mortality  
18 rates in patients with a cirrhotic liver who undergo surgery [5,6]. However,  
19 the impact of preoperative nutritional status as well as of nutritional  
20 intervention on postoperative infectious complications in LT remains  
21 controversial [7-10], especially in patients undergoing living donor LT  
22 (LDLT). Patients with advanced cirrhosis characteristically show a decrease

in plasma concentrations of branched-chain amino acids (BCAAs). These BCAAs serve not only as an essential substrate in the synthesis of body proteins, but also act as an important regulator of protein turnover. Moreover, BCAAs have beneficial effects on hepatic encephalopathy through the promotion of ammonia detoxification and the correction of plasma amino acid imbalance, liver regeneration, and hepatic cachexia in patients with liver diseases [11]. Improving systemic conditions, including nutritional status, to the greatest extent possible before LT facilitates early postoperative recovery. Supplementation with a BCAA-enriched nutrient mixture is reportedly beneficial not only for patients with liver cirrhosis, but also for patients undergoing hepatectomy [12-15]. However, the value of pretransplant BCAA supplementation remains unclear. The aim of the present study was therefore to examine pre- and perioperative predictors including nutritional factors such as BCAA supplementation for posttransplant infectious complications so that a strategy can be established to improve short-term outcomes after LDLT.

## Methods

The present report retrospectively analyzed data from 100 consecutive adult patients (46 males and 54 females) aged  $\geq 18$  (range, 18 – 69; median, 56) years who underwent LDLT at Kyoto University Hospital between February 2008 and February 2010 after introducing the nutritional

assessment described below. The Model for End-stage Liver Disease (MELD) score was 19 (range, 7 – 46), 32 patients were ABO incompatible and 68 were identical or compatible. The indications for LT were hepatocellular carcinoma (n = 33), followed by hepatocellular diseases such as hepatitis B or C virus-associated liver cirrhosis (n = 19), progressive intrahepatic cholestatic diseases including primary biliary cirrhosis and primary sclerosing cholangitis (n = 13), fulminant hepatic failure (n = 11) and other causes (n = 24). The patients provided written informed consent before the start of the study, which was approved by the Ethics Committee of Kyoto University and conducted in accordance with the Declaration of Helsinki of 1975 as revised in 1996.

We introduced body composition analysis using multifrequency bioelectrical impedance with eight tactile electrodes (InBody 720; Biospace, Tokyo, Japan) in February 2008 for patients undergoing LT. Patients fast for at least 3 hours and void immediately before starting the analysis. Various parameters are automatically measured, including body mass index, intra- and extracellular water, body fat mass, protein, and body cell mass (BCM), which is the sum of intracellular fluid and protein and a reliable parameter of nutritional status. The BCM is automatically calculated by the InBody 720 for each patient and displayed as a normal range (e.g. 23.0 ~ 28.1 kg). Low and high BCM values are below the lower limit and above the upper limit, respectively. Ten patients who could not undergo



preoperative InBody 720 examination due to undergoing emergency surgery were excluded from the BCM analysis.

Preoperative nutritional therapy was administered for about 2 weeks before LDLT at the discretion of the surgeon or attending physician after admission to our department. The therapy consisted of a nutrient mixture enriched with branched-chain amino acids (BCAA; Aminoleban EN; Otsuka Pharmaceutical Co., Tokyo, Japan), BCAA nutrients (Livact; Ajinomoto Pharma Co., Tokyo, Japan) or none. Thirty-seven of the patients received the preoperative BCAA-enriched nutrient mixture (100 g/day), 28 received BCAA nutrients (12.45 g/day), and 35 received no nutritional therapy. Dieticians adjusted the type and amount of food for each patient to maintain a total caloric intake of 35 - 40 kcal/kg and a protein intake of 1.2 to 1.5 g/kg including BCAA nutrients according to the guidelines of the European Society of Parenteral and Enteral Nutrition.

The selection criteria for the recipients as well as surgical techniques for recipient operations have been described in detail elsewhere [16-18]. Immunosuppressive therapy usually consisted of tacrolimus or cyclosporine and low-dose steroids as described elsewhere [18,19].

We examined preoperative risk factors (including preoperative nutritional parameters) for posttransplant sepsis, posttransplant bacteremia and in-hospital death due to infection. Data regarding the following recipient variables for each patient were analyzed: age of recipient, gender,

original disease underlying the need for LT, ABO compatibility, Child-Pugh classification, MELD score, graft type (right or left lobe), graft-recipient weight ratio (GRWR), operative duration, operative blood loss, pretransplant BCM, and preoperative nutritional therapy. We defined conditions fulfilling the diagnostic criteria for systemic inflammatory response syndrome with infections including blood, urine and pulmonary infection as sepsis [20]. Infections were defined using the criteria proposed by the Centers for Disease Control and based on reports regarding liver transplant patients [21]. Isolation of bacteria other than common skin contaminants from a single blood culture in the presence of clinical symptoms or of infection was considered bacteremia. When caused by common skin contaminants, bacteremia was considered significant only if an organism was isolated from two blood cultures and clinical signs of infection were evident.

## Statistical analysis

Categorical variables were compared using the  $\chi^2$  test or Fisher's exact test where appropriate. Any variable identified as significant ( $P < 0.05$ ) or with  $P < 0.10$  in univariate analyses using the above tests was considered a candidate for multivariate analysis using multiple logistic regression models. A  $P$  value of  $< 0.05$  was considered significant. All data were statistically analyzed using JMP 5.0.1 software.

1

2   **Results**3   **Posttransplant sepsis**

4   Univariate analysis showed that age <60 years, MELD score  $\geq 20$ , low  
5   pretransplant BCM and the absence of preoperative supplementation with  
6   the BCAA-enriched nutrient mixture were of prognostic significance for  
7   posttransplant sepsis (Table 1). Multivariate analysis revealed that low  
8   pretransplant BCM ( $p = 0.032$ ) and no preoperative BCAA-enriched  
9   nutrient supplementation ( $p = 0.020$ ) were of independent prognostic  
10   significance for posttransplant sepsis (Table 2).

11

12   **Bacteremia**

13   Age <60 years, Child-Pugh classification C, perioperative blood loss  $\geq 10$  L  
14   and no preoperative BCAA-enriched supplementation were risk factors for  
15   bacteremia (Table 3). Multivariate analysis revealed that Child-Pugh  
16   classification C ( $p = 0.002$ ) and perioperative blood loss  $\geq 10$  L ( $p = 0.018$ )  
17   were independent risk factors for posttransplant bacteremia (Table 4).

18

19   **In-hospital death due to infection**

20   Age <60 years, Child-Pugh classification C and low preoperative BCM  
21   were significant risk factors for in-hospital death due to infection (Table 5).

1 Multivariate analysis showed that only low preoperative BCM ( $p = 0.004$ )  
2 was an independent risk factor (Table 6).

## 3 4 **Discussion**

5 The present study examined risk factors affecting three types of infectious  
6 complications after LDLT. We identified the independent risk factors as  
7 Child-Pugh classification C, massive perioperative blood loss, low  
8 pretransplant BCM and the absence of preoperative supplementation with a  
9 BCAA-enriched nutrient mixture. Decompensated liver cirrhosis indicated  
10 by Child-Pugh classification C is usually accompanied by deteriorated  
11 immune function and nutritional status at the time of LT. Child-Pugh  
12 classification C was thus undoubtedly a risk factor of postoperative  
13 infection, which is in line with the results of our recent report [22]. Massive  
14 perioperative bleeding is an established factor for postoperative  
15 complications of digestive surgery as well as LT and massive blood loss is  
16 usually associated with massive blood transfusion. Homologous blood  
17 transfusion has adverse effects such as a risk of infection and  
18 graft-versus-host disease. This detrimental effect is supposed to be caused  
19 by nonspecific immunosuppression such as decreased CD4/CD8 ratios  
20 [23,24] and natural killer cell activity [25,26]. We recently reported that  
21 low pretransplant BCM and the absence of preoperative BCAA-enriched  
22 supplementation are closely associated with postoperative sepsis [27]. The

1 present findings supported not only our previous results but also  
2 demonstrated the powerful impact of pretransplant nutritional conditions  
3 and preoperative treatment with BCAA-enriched nutrient mixture on  
4 infectious complications.

5 The reason for the beneficial effects of pretransplant BCAA  
6 supplementation, however, remains unclear. One possible explanation is  
7 improvement in pretransplant nutritional status. Some nutritional  
8 parameters, such as prealbumin, total lymphocyte count, and  
9 BCAA/tyrosine ratio, were significantly improved by pretransplant  
10 nutritional intervention including BCAA-enriched nutrient mixture (in  
11 submission). Another possible reason is the improvement of the immune  
12 system. Bassit et al. reported that BCAA supplementation improves the  
13 ability of peripheral blood mononuclear cells to proliferate in response to  
14 mitogens after long distance intense exercise [28]. Lorenzo et al. reported  
15 that septic patients receiving a high-BCAA preparation showed decreased  
16 mortality and improved nutritional parameters [29]. In patients with  
17 advanced liver cirrhosis, Kakazu et al. showed that elevating extracellular  
18 concentration of BCAAs ex vivo improved the function of myeloid  
19 dendritic cells [30]. Our results suggest that preoperative BCAA-enriched  
20 supplementation can help to prevent postoperative sepsis through  
21 nutritional and immune improvement, although a randomized controlled  
22 study is required to confirm this hypothesis. Taken together with our

findings demonstrating that the absence of posttransplant enteral nutrition is a risk factor affecting in-hospital mortality after LT [1], perioperative nutritional treatment represents a promising strategy for improving short-term outcomes after LT.

Based on the current findings, we considered establishing an interventional strategy against these risk factors to prevent posttransplant infectious complications. Child-Pugh classification C itself is an indication for LT. In contrast, massive blood loss, pretransplant low BCM and the absence of preoperative BCAA-enriched supplementation are factors that can be altered to some extent. Blood loss can be reduced by more careful surgical maneuvering and the frequent application of hemostatic devices during dissection of the liver from surrounding ligaments and the inferior vena cava. The sum of intracellular fluid and body protein, BCM, is considered a highly reliable parameter of nutritional status. Especially for patients undergoing LT who usually have abundant extracellular fluid such as edema and ascites, BCM can assess their nutritional status more accurately than other nutritional parameters including body mass index and lean body mass. Low BCM in patients with cirrhosis suggests a decrease in skeletal muscle volume, which could interfere with early postoperative mobilization and result in pulmonary complications including aspiration pneumonia and atelectasis. Therefore, we have recently introduced a pretransplant rehabilitation program to encourage early postoperative

1 mobilization and avert pulmonary dysfunction. Since LDLT is an elective  
2 procedure that differs from deceased donor LT, a pretransplant  
3 rehabilitation program can be implemented until the day of transplant.

4 **The prevalence of metabolic disorders including metabolic syndrome**  
5 **on liver–transplant population has recently attracted attention [31-33].**

6 **The prevalence of metabolic syndrome in post-LT patients is**  
7 **significantly higher than that estimated in the general population and**  
8 **metabolic syndrome is associated with an increased risk of major**  
9 **vascular events and long-term fibrosis progression. Therefore,**  
10 **prevention of post-LT metabolic syndrome would also be a crucial**  
11 **objective of perioperative nutritional treatment.**

12 Supplementation with a BCAA-enriched nutrient mixture is reportedly  
13 beneficial not only for patients with liver cirrhosis but also for patients  
14 undergoing hepatectomy [23-26]. However, the value of pretransplant  
15 BCAA supplementation has remained unclear. Our results suggest that  
16 preoperative BCAA-enriched supplementation can help to prevent  
17 postoperative sepsis, although a randomized controlled study is required to  
18 confirm this notion. Taken together with our findings demonstrating that  
19 the absence of posttransplant enteral nutrition is a risk factor affecting  
20 in-hospital mortality after LT [1], perioperative nutritional treatment should  
21 be a promising strategy to improve short-term outcomes after LT.

22

## 1    **Conclusion**

2    Preoperative nutritional status, supplementation with a nutrient mixture  
3    enriched with BCAAs and massive operative blood loss were closely  
4    associated with the occurrence of posttransplant infectious complications.  
5    Perioperative management including nutritional therapy is needed to  
6    improve short-term outcomes after LT.

7

8



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2

- 1 Table 1. Univariate analysis of factors affecting posttransplant sepsis.
- 2
- 3 Table 2. Multivariate analysis of factors affecting posttransplant sepsis.
- 4
- 5 Table 3. Univariate analysis of factors affecting posttransplant bacteremia.
- 6
- 7 Table 4. Multivariate analysis of factors affecting posttransplant
- 8 bacteremia.
- 9
- 10 Table 5. Univariate analysis of factors affecting in-hospital death due to
- 11 infection.
- 12
- 13 Table 6. Multivariate analysis of factors affecting in-hospital death due to
- 14 infection.

Table 1. Univariate analysis of factors affecting posttransplant sepsis

	Variable	Incidence of event	P value
Age (y)	<60 (n=68)	72%	0.001
	≥60 (n=32)	38%	
Gender	Male (n=46)	52%	0.051
	Female (n=54)	70%	
Original disease	HCC (n=34)	50%	0.460
	HBV/HCV (n=19)	68%	
	PBC/PSC (n=20)	70%	
	FHF (n=8)	75%	
	Others (n=19)	58%	
ABO blood type	Compatible (n=61)	57%	0.166
	Incompatible (n=39)	71%	
Child-Pugh	A, B (n=39)	51%	0.112
	C (n=61)	67%	
MELD score	<20 (n=55)	51%	0.021
	≥20 (n=45)	73%	
GRWR	<0.8% (n=28)	50%	0.163
	≥0.8% (n=72)	65%	



Graft	Rt (n=57)	61%	0.924
	Lt (n=43)	60%	
Operative time	<12h (n=25)	68%	0.403
	≥12h (n=75)	59%	
Operative blood loss	<10L (n=65)	58%	0.476
	≥10L (n=35)	66%	
Preoperative BCM	Low (n=24)	83%	0.002
	Normal or high (n=64)	48%	
Preoperative BCAA enriched nutrient mixture			0.001
With (n=37)		38%	
Absent (n=63)		73%	

HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; PBC, primary biliary cirrhosis; PSC, primary sclerosing cirrhosis; FHF, fulminant hepatic failure; MELD, model for end-stage liver disease; GRWR, graft to recipient weight ratio; BCM, body cell mass; BCAA, branched-chain amino acids.

**Table 2. Multivariate analysis of factors affecting posttransplant sepsis**

Variable	Odds ratio	95% CI	<i>P</i>
Preoperative low BCM	4.633	1.493-17.701	0.032
Absence of preoperative BCAA enriched nutrient mixture	3.201	1.202-7.849	0.020

**Table 3. Univariate analysis of factors affecting posttransplant bacteremia**

	Variable	Incidence of event	<i>P</i> value
Age (y)	<60 (n=68)	51%	0.011
	≥60 (n=32)	25%	
Gender	Male (n=46)	39%	0.470
	Female (n=54)	46%	
Original disease	HCC (n=34)	41%	0.880
	HBV/HCV (n=19)	53%	
	PBC/PSC (n=20)	45%	
	FHF (n=8)	38%	
	Others (n=19)	37%	
ABO blood type	Compatible (n=61)	39%	0.245
	Incompatible (n=39)	52%	
Child-Pugh	A, B (n=39)	23%	0.001
	C (n=61)	56%	
MELD score	<20 (n=55)	35%	0.059
	≥20 (n=45)	53%	
GRWR	<0.8% (n=28)	39%	0.639
	≥0.8% (n=72)	44%	

Graft	Rt (n=57)	47%	0.309
	Lt (n=43)	33%	
Operative time	<12h (n=25)	40%	0.726
	≥12h (n=75)	44%	
Operative blood loss	<10L (n=65)	34%	0.012
	≥10L (n=35)	60%	
Preoperative BCM	Low (n=24)	54%	0.093
	Normal or high (n=64)	34%	
Preoperative BCAA enriched nutrient mixture			0.015
	With (n=37)	26%	
	Absent (n=63)	52%	

HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; PBC, primary biliary cirrhosis; PSC, primary sclerosing cirrhosis; FHF, fulminant hepatic failure; MELD, model for end-stage liver disease; GRWR, graft to recipient weight ratio; BCM, body cell mass; BCAA, branched-chain amino acids.

**Table 4. Multivariate analysis of factors affecting posttransplant bacteremia**

Variable	Odds ratio	95% CI	<i>P</i>
Child-Pugh C	4.253	1.731-11.294	0.001
Operative blood loss ≥10L	2.983	1.229-7.541	0.018

**Table 5. Univariate analysis of factors affecting in-hospital death due to infection**

	Variable	Incidence of event	<i>P</i> value
Age (y)	<60 (n=68)	19%	0.017
	≥60 (n=32)	3%	
Gender	Male (n=46)	17%	0.369
	Female (n=54)	11%	
Original disease	HCC (n=34)	6%	0.462
	HBV/HCV (n=19)	21%	
	PBC/PSC (n=20)	20%	
	FHF (n=8)	13%	
	Others (n=19)	16%	
ABO blood type	Compatible (n=61)	13%	0.684
	Incompatible (n=39)	16%	
Child-Pugh	A, B (n=39)	5%	0.030
	C (n=61)	20%	
MELD score	<20 (n=55)	9%	0.118
	≥20 (n=45)	20%	
GRWR	<0.8% (n=28)	7%	0.192
	≥0.8% (n=72)	17%	

Graft	Rt (n=57)	16%	0.550
	Lt (n=43)	12%	
Operative time	<12h (n=25)	8%	0.293
	≥12h (n=75)	16%	
Operative blood loss	<10L (n=65)	11%	0.213
	≥10L (n=35)	20%	
Preoperative BCM	Low (n=24)	29%	0.003
	Normal or high (n=64)	5%	
Preoperative BCAA enriched nutrient mixture			0.884
	With (n=37)	14%	
	Absent (n=63)	15%	

HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; PBC, primary biliary cirrhosis; PSC, primary sclerosing cirrhosis; FHF, fulminant hepatic failure; MELD, model for end-stage liver disease; GRWR, graft to recipient weight ratio; BCM, body cell mass; BCAA, branched-chain amino acids.

**Table 6. Multivariate analysis of factors affecting in-hospital death due to infection**

Variable	Odds ratio	95% CI	<i>P</i>
Preoperative low BCM	8.372	2.092-42.181	0.004